American Floral Endowment | Research Internships Scholarships Education

Funding the Future of Floriculture

2022 Annual Progress Report

Use of CRISPR to Develop Powdery Mildew Resistance in Gerbera

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(1) Industry needs that were addressed: This project investigates the use of gene editing to improve floral crops. The ability to confer disease resistance through CRISPR technology is being examined in gerbera and this approach should be applicable to other floral species.

(2) Research summary and benefits to industry: This research will benefit industry by developing gene editing methods for floral crop improvement. Our focus was on gerbera cultivars in the marketplace, not a model genotype. While this complicated the research, the results should be more relevant to the implementation of this technology by industry. In the last year, our efforts were concentrated on four areas: shoot multiplication, induction of organogenesis and somatic embryogenesis, genetic transformation, and CRISPR construct design.

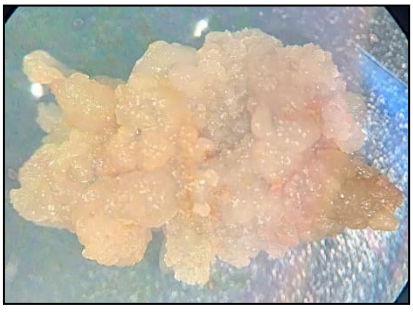
(a) <u>Shoot multiplication</u>:

A source of leaf blades and petioles was required for tissue culture and transformation experiments. We developed and maintained sterile cultures of four gerbera cultivars: 'Flori Line Maxi Yellow', Revolution 'Bicolor Red Lemon', Garvinea "Sweet Love", and Majorette 'Pink Halo'. The optimal medium for shoot multiplication of all four cultivars was determined to be Murishige and Skoog basal medium and vitamins (MS) supplemented with 0.1 mg/L IBA, 1.0 mg/L BAP, 0.5 mg/L glutamine, 30 g/L sucrose, and 3 g/L gelrite. Lower light conditions (8 µE m^{-2} s^{-1}) were found to promote more vigorous shoot production.

(b) <u>Organogenesis and somatic embryogenesis</u>: Efficient methods for regenerating gerbera from tissue culture are required to produce transgenic and gene-edited plants. The tissue culture variables that we investigated to induce shoots included the cultivar, the type of explant, and the combination of plant growth regulators (PGRs). In the past year, we focused our research primarily on petioles and leaf blades of two cultivars, 'Flori Line Maxi Yellow' and Majorette 'Pink Halo'. The PGRs examined included indole acetic acid (IAA), indole butyric acid (IBA), benzylaminopurine (BAP), and thidiazuron (TDZ), individually or in combinations. For shoot organogenesis, the most effective induction treatment was with leaf blades and petioles exposed to 0.1 mg/L IBA and 2.0 BAP mg/L. This PGR combination was used in subsequent gene transfer experiments.

Majorette 'Pink Halo' organogenesis

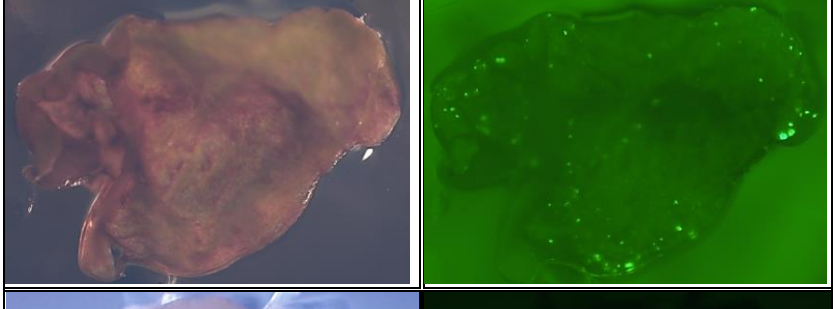
Somatic embryogenesis is an alternative approach to regeneration that we are pursuing because embryogenic tissue will be a good target for biolistics and transgene-free gene editing. Leaf explants of 'Flori Line Maxi Yellow' and Majorette 'Pink Halo' were exposed to solid MS medium containing three levels of 2,4-D (1.0, 2.0 and 3.0 mg/L) or picloram (0.05, 0.1 and 0.5 mg/L). After two months, the explants were transferred to the same medium, in either solid or liquid form. Potentially embryogenic callus was induced from Majorette 'Pink Halo' with 2,4-D.



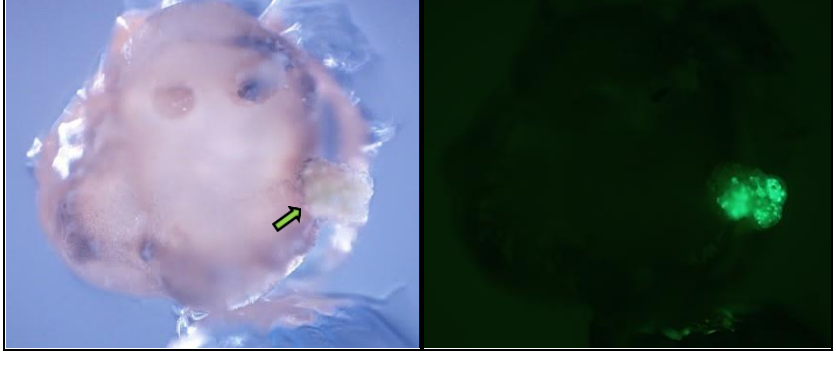
SE callus induction from Majorette 'Pink Halo'

(d) Genetic transformation: Despite reports in the literature of gerbera transformation with the *NPTII* selectable marker, we found that kanamycin selection was not effective for the cultivars with which we were working. The hygromycin B phosphotransferase gene (*hph*), on the other hand, yielded positive results more consistently. Sensitivity tests with hygromycin at 0, 10, 15, and 20 mg/L indicated that the antibiotic was effective at 10 mg/L. *HPH* and *GFP* transgenes were introduced into leaf explants of 'Flori Line Maxi Yellow' using *Agrobacterium tumefaciens* C58 in a series of multi-factor experiments. GFP expression could be observed in multiple sites across leaf explants after two weeks, indicating stable transformation. In the presence of 0.1 mg/L IBA and 2.0 BAP mg/L, regenerating tissue was obtained that expressed GFP.

Leaf blades and petioles of 'Flori Line Maxi Yellow' exposed to hygromycin at 0 (left) and 10 ml/L (right)



Multiple sites of gene transfer showing GFP expression



GFP-expressing tissue regenerating in the presence of 20 mg/L hygromycin.

(d) CRISPR constructs: After GFP-transformed gerbera plants are validated by molecular analysis, CRISPR constructs to silence genes for phytoene desaturase (PDS) and MLO will be introduced. Gerbera *PDS* is being used as a target to optimize gene editing because its mutagenesis causes an easily scorable, albino phenotype. A 768 bp portion of *GhPDS* was used to design sequence guide RNAs (sgRNAs) for insertion into a CRISPR construct containing the *Cas9* endonuclease. Dr. Zhanao Deng (UF/IFAS) indicated that he could provide gerbera sequence data for designing sgRNAs to target the correct *MLO* gene.

(3) Provide the objectives to be completed in the coming year:

- *Regenerate gerbera plants transformed with GFP and HPH*: *GFP*-transformed shoots will be rooted *in vitro*, transferred to soil, and acclimated to ambient conditions. PCR analysis will be conducted to confirm the presence of *GFP* and *HPH*.

- *Develop gerbera plants with silenced GhPDS*: A CRISPR construct with sgRNAs for *GhPDS* will be introduced into *Agrobacterium* strain C58. Leaves from cultivar 'Flori Line Maxi Yellow' will be subjected to *Agrobacterium*-mediated transformation and hygromycin selection. The efficiency of gene editing of *GhPDS* will be measured by the number of albino shoots produced and confirmed by molecular analysis of *GhPDS* mutations.

- *Develop gerbera plants with silenced GhMLO:* Sequence data for gerbera *MLO* genes will be obtained from the Deng lab and used to design sgRNAs. Leaves from cultivar 'Flori Line Maxi Yellow' will be transformed with *MLO* CRISPR constructs and plants will be regenerated in the presence of hygromycin. *GhMLO* mutations will be characterized by molecular analysis. Plants with mutations that result in *GhMLO* silencing will be propagated and tested for powdery mildew resistance, although fungal resistance assays will likely be completed after the grants ends.

- *Gerbera somatic embryogenesis*: Research will continue on the optimization of media for the induction of somatic embryogenesis (SE) and the regeneration of somatic embryos. If an efficient SE system is developed, transformation via biolistics will be investigated as a means to obtain transgene-free, gene-edited plants.

(4) Provide list of publications and presentations in the past year of this project. Please provide copies of all published information related to this project.
None to date

(5) Plans for publications and presentations in the coming year.
Publications
a. Regeneration of transgenic gerbera plants expressing GFP
b. Gene editing of gerbera to silence phytoene desaturase
c. Resistance of gerbera to powdery mildew by gene editing of *MLO* (after grants ends)

Presentations
a. Plant Biology national meeting (Savannah, GA): *Gene editing of gerbera*
b. American Society for Horticultural Science national meeting (Orlando, FL): *Powdery mildew resistance in gerbera through gene editing*
c. UGA Plant Center retreat (Helen, GA) *Tissue culture and transformation of gerbera*

We will acknowledge the AFE in all publications and presentations.

(6) What is the compelling reason(s) the Endowment should continue funding your research?

Progress on gerbera gene editing research has been slower than expected due to Covid restrictions (in year 1) and the recalcitrance of gerbera cultivars to genetic transformation. We have found that selection for transgenic tissue with the antibiotic hygromycin instead of kanamycin is giving us more consistent expression of the *GFP* transgene. In addition to the alternative selectable marker, we have determined the most effective *Agrobacterium* strain and plant growth regulator combinations for producing transgenic plants from the gerbera cultivars with which we are working. We are now in a position to introduce CRISPR constructs, first for the model gene *GhPDS*, then for the powdery mildew susceptibility gene *GhMLO*.

The AFE projects provides funding for half of a postdoc's time. To assist her, we have added two PhD students that are funded by other sources. One of these students is focusing on gerbera tissue culture, including organogenesis and somatic embryogenesis. The other student has MS experience with CRISPR and is responsible for designing sgRNAs and developing CRISPR constructs. With this team in place, we expect to produce gene-edited gerbera plants by the conclusion of this project.